

Appl. No. 10/020,786
Amtd. dated November 14, 2003
Response to Office Action mailed on June 17, 2003

Patent Docket No. P1793R1

Amendments to the Claims:

What is claimed is:

1. (currently amended) A polynucleotide molecule encoding an immunoglobulin, said polynucleotide molecule comprising (1) a first promoter and a first cistron forming a first promoter-cistron pair and (2) a second promoter and a second cistron forming a second promoter-cistron pair, wherein the first cistron of said first promoter-cistron pair comprises a first translational initiation region (TIR-L) operably linked to a nucleic acid sequence encoding an immunoglobulin light chain and the second cistron of said second promoter-cistron pair comprises a second translational initiation region (TIR-H) operably linked to a nucleic acid sequence encoding an immunoglobulin heavy chain, wherein upon expression of said polynucleotide in a prokaryotic host cell, the secreted light and heavy chains are folded and assembled to form a biologically active immunoglobulin.
2. (original) The polynucleotide molecule of claim 1, wherein the first and second promoters are prokaryotic promoters selected from the group consisting of phoA, tac, lpp, lac-lpp, lac, ara, trp, trc and T7 promoters.
3. (original) The polynucleotide molecule of claim 2, wherein both promoters are PhoA promoters.
4. (original) The polynucleotide molecule of claim 1, wherein each of the TIR-L and TIR-H comprises a prokaryotic secretion signal sequence or variant thereof.
5. (original) The polynucleotide molecule of claim 4, wherein the prokaryotic secretion signal sequence is selected from the group consisting of STII, OmpA, PhoE, LamB, MBP and PhoA secretion signal sequences.
6. (original) The polynucleotide molecule of claim 1, wherein the TIR-L and TIR-H provide approximately equal translational strengths.
7. (original) The polynucleotide molecule of claim 6, wherein the relative translational strength combination is about (1-TIR-L, 1-TIR-H).

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8. (original) A recombinant vector for expressing an immunoglobulin in a prokaryotic host cell, said vector comprising the polynucleotide molecule of claim 1.
9. (original) A prokaryotic host cell comprising the recombinant vector of claim 8.
10. (original) The prokaryotic host cell of claim 9 which is a gram-negative bacterial cell.
11. (original) The host cell of claim 10 which is *E. coli*.
12. (original) The host cell of claim 11, further comprising a polynucleotide encoding at least one prokaryotic polypeptide selected from the group consisting of DsbA, DsbC, DsbG and FkpA.
13. (original) The host cell of claim 12, wherein the polynucleotide encodes both DsbA and DsbC.
14. (original) The host cell of claim 11, wherein the *E. coli* is of a strain deficient in endogenous protease activities.
15. (original) The host cell of claim 14, wherein the genotype of the *E. coli* strain lacks *degP* and *prc* genes and harbors a mutant *spr* gene.
16. (currently amended) A process for producing a biologically active immunoglobulin in a prokaryotic host cell, said process comprising expressing in the host cell a polynucleotide comprising (1) a first promoter and a first cistron forming a first promoter-cistron pair and (2) a second promoter and a second cistron forming a second promoter-cistron pair, wherein the first cistron of said first promoter-cistron pair comprises a first translational initiation region (TIR-L) operably linked to a nucleic acid sequence encoding an immunoglobulin light chain and the second cistron of said second promoter-cistron pair comprises a second translational initiation region (TIR-H) operably linked to a nucleic acid sequence encoding an immunoglobulin heavy chain, wherein upon expression of said polynucleotide, the secreted light chain and heavy chain are folded and assembled to form a biologically active immunoglobulin; and recovering said immunoglobulin.
17. (original) The process of claim 16, wherein the first and the second promoters are prokaryotic promoters selected from the group consisting of *phoA*, *tac*, *lpp*, *lac-lpp*, *lac*, *ara*, *trp*, *trc* and *T7*

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promoters.

18. (original) The process of claim 17, wherein both the first and the second promoters are PhoA promoters.
19. (original) The process of claim 16, wherein each of the TIR-L and TIR-H comprises a prokaryotic secretion signal sequence or variant thereof.
20. (original) The process of claim 19, wherein the prokaryotic secretion signal sequence is selected from the group consisting of STII, OmpA, PhoE, LamB, MBP and PhoA secretion signal sequences.
21. (original) The process of claim 16, wherein the TIR-L and TIR-H provide approximately equal translational strengths.
22. (original) The process of claim 21, wherein the relative translational strength combination is about (1-TIR-L, 1-TIR-H).
23. (original) The process of claim 16, wherein the prokaryotic host cell is *E. coli*.
24. (original) The process of claim 16, further comprising expressing in the prokaryotic host cell a polynucleotide encoding at least one prokaryotic polypeptide selected from the group consisting of DsbA, DsbC, DsbG and FkpA.
25. (original) The process of claim 24, wherein the polynucleotide encodes both DsbA and DsbC.
26. (original) The process of claim 23, wherein the *E. coli* is of a strain deficient in endogenous protease activities.
27. (original) The process of claim 26, wherein the genotype of the *E. coli* lacks *degP* and *prc* genes and harbors a mutant *spr* gene.
28. (Withdrawn) An aglycosylated full length antibody produced by a process according to claim 16.

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29. (Withdrawn) The aglycosylated full length antibody of claim 28, wherein the immunoglobulin is a multispecific antibody.
30. (Withdrawn) The aglycosylated full length antibody of claim 28, which is a non-human antibody.
31. (Withdrawn) The aglycosylated full length antibody of claim 30, wherein the non-human antibody is humanized.
32. (Withdrawn) The aglycosylated full length antibody of claim 28, which is a human antibody.
33. (Withdrawn) An immunoconjugate comprising the aglycosylated full length antibody of claim 28 conjugated with a cytotoxic agent.
34. (Withdrawn) The immunoconjugate of claim 33, wherein the cytotoxic agent is selected from the group consisting of a radioactive isotope, a chemotherapeutic agent and a toxin.
35. (Withdrawn) The immunoconjugate of claim 34, wherein the toxin is selected from the group consisting of calichemycin, maytansine and trichothene.
36. (Withdrawn) A composition comprising the aglycosylated full length antibody of claim 28 and a carrier.
37. (Withdrawn) The composition of claim 36, wherein the carrier is pharmaceutically acceptable.
38. (Withdrawn) A composition comprising the immunoconjugate of claim 33 and a carrier.
39. (Withdrawn) The composition of claim 38, wherein the carrier is pharmaceutically acceptable.
40. (Withdrawn) An article of manufacture comprising a) a container and a composition contained therein, wherein the composition comprises an aglycosylated full length antibody of claim 28;

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and b) instruction for using said composition.

41. (Withdrawn) An article of manufacture comprising a) a container and a composition contained therein, wherein the composition comprises an immunoconjugate according to claim 33; and b) instruction for using said composition.

This listing of claims will replace all prior versions, and listings, of claims in the application: